Salmonella Penetration of Egg Shells and Proliferation in Broiler Hatching Eggs—A Review

N. A. Cox,[1] M. E. Berrang, and J. A. Cason

USDA, Agricultural Research Service-Russell Research Center, P.O. Box 5677, Athens, Georgia 30604-5677

ABSTRACT The presence of salmonellae in fertile broiler hatching eggs has been clearly identified as a critical control point in the salmonellae contamination of broiler chickens. This paper reviews the published research studies on a) the penetration and proliferation of salmonellae in hatching eggs, b) the consequences of this contamination on the contamination of the final product, and c) the egg's defenses against invading salmonellae. A better understanding of the material in this review paper will assist poultry researchers and the poultry industry in continuing to make progress in reducing and eliminating salmonellae from fertile hatching eggs, hatcheries, and breeder flocks.

(*Key words*: *Salmonella*, egg, broiler, penetration, proliferation)

2000 Poultry Science 79:1571–1574

THE ORGANISM

The genus *Salmonella* consists of facultative, gram-negative rods in the family Enterobacteriaceae. *Salmonella* are relatively small bacteria measuring about 0.5 μm by 2 to 3 μm; most strains are motile with peritrichous flagella. Typical *Salmonella* can be differentiated from other members of the family by lack of fermentation of lactose, fermentation of glucose with production of gas, and production of H_2S from thiosulfate. *Salmonella* can grow across a wide range of temperatures, the optimum usually being 37 C. (Bierer et al., 1961).

PATHOGENESIS

Salmonella is well known for its ability to cause disease in humans. When associated with food-borne outbreaks, *Salmonella* infection usually leads to gastroenteritis. Ingestion of as few as one to ten cells of a disease-causing strain of *Salmonella* can lead to penetration of the epithelial lining of the small intestine. Growth in the underlying tissue causes destruction of the epithelial lining. After an incubation period of 8 to 72 h, the victim usually suffers diarrhea and abdominal pain with or without fever. The disease is usually self-limiting in healthy adults, but can be life-threatening in small children and the elderly or infirm (Zwadyk, 1992; D'Aoust, 1997).

SALMONELLA IN EGGS

Much of the evidence of a link between *Salmonella* and eggs comes from the table egg industry. Numerous outbreaks of *Salmonella* have been reported, usually involving the consumption of raw or undercooked eggs (Anonymous, 1990; Anonymous, 1996a, 1996b). In the table egg industry, the strain of concern is *Salmonella enteritidis*. Henzler et al. (1994) traced egg association in outbreaks back to layer houses. Upon sampling, the same phage type of *S. enteritidis* seen in the outbreak was cultured from eggs gathered at the farm.

When most people think of salmonellae and eggs they think about *S. enteriditis* and table eggs. However, human salmonellosis can also be traced to broilers. The penetration of fertile broiler hatching eggs by the other paratyphoid *Salmonella* serovars is important to human health. The presence of salmonellae in hatching eggs has been identified as a critical point in the salmonellae contamination of broiler chickens.

ORIGIN OF *SALMONELLA*-CONTAMINATED TABLE EGGS AND FERTILE HATCHING EGGS

There are two main schools of thought regarding entry of salmonellae into hatching eggs. The vertical transmission theory states that salmonellae come from an infected hen. The horizontal transmission theory states that salmonellae invade the egg through the shell after the egg is laid. In truth, both routes are probably involved.

Vertical Contamination

Many published reports show that *Salmonella*-contaminated eggs can be produced by artificially inoculating the hen. Timoney et al. (1989) showed that oral inoculation of laying hens could produce infection of the reproductive

Received for publication April 10, 2000.
Accepted for publication June 30, 2000.
[1]To whom correspondence should be addressed: ncox@ars.usda.gov.

tract. A challenge of 10^6 cells caused the ovary and oviduct to become infected. The egg production rate was unaffected, and *Salmonella* was not detected in all fecal samples; thus an infection with salmonellae may not always be easily detectable on the farm. The yolks of 10% of the eggs laid by these hens were contaminated with *S. enteritidis*. Another study showed that inoculation with higher doses (10^8 cells per hen) led to a noticeable drop in egg production and signs of pathogenesis in the hen (Shivaprasad et al., 1990). However, these researchers confirmed Timoney's findings that inoculation with lower numbers did not cause noticeable signs in the hens, but did lead to contaminated eggs.

By inoculating hens using different routes, Miyamoto et al. (1997) were able to examine where contamination was occurring in the oviduct. They found that intravenous inoculation caused colonization of the ovary and contamination of forming eggs while in the oviduct. Intravaginal inoculation led to colonization of only the lower portions of the oviduct, but internally contaminated eggs were produced. Thus, some internal contamination of eggs may be coming from the lower oviduct and may actually be due to penetration of the shell in the oviduct and not colonization of the ovary. Keller et al. (1995) also reported the importance of lower oviduct contamination in the production of infected eggs. They found that while forming, eggs may be contaminated due to the colonization of an inoculated hen's ovary. This contamination is sometimes lessened as the egg progresses through the oviduct. Upon entering a contaminated lower oviduct, the egg can then be recontaminated (Keller et al., 1995). Therefore, the egg is subject to challenge in both the upper and lower oviduct.

Gast and Beard (1990) showed that noninoculated hens can become contaminated and lay infected eggs just by being exposed to inoculated pen mates, which raises the question of how birds may be contaminated in farm conditions and blurs the line between vertical and horizontal transmission. It has been shown that the hen's ovary can be colonized with *S. enteritidis* through airborne inoculation (Baskerville et al., 1992). Humphrey et al. (1992) suggested a conjunctival route of infection in a situation in which airborne *Salmonella* is prevalent. Delivery of about 100 cells to the eye of laying hens produced *Salmonella* infection of the ovary and oviduct.

It is apparent that the reproductive tract of a laying hen can be colonized experimentally and there are reports detailing various methods to detect low numbers of *Salmonella* that may be present in a few eggs (Gregory, 1948; Gast and Beard, 1992; Gast, 1993). When examining naturally contaminated hens, the incidence of *S. enteritidis* in eggs is low. Poppe et al. (1992) found that less than 0.065% of eggs tested (two positive samples from 16,000 eggs) were positive. Humphrey et al. (1989, 1991) also found low numbers of *S. enteritidis*-contaminated eggs when sampling naturally contaminated flocks; however, these researchers also found eggs with other strains of *Salmonella*.

There are many sources of salmonellae for the hens on the breeder farm, and it is evident that eggs can be contaminated before lay. However, contamination of the egg after lay (horizontal transmission) is also a concern.

Horizontal Contamination

The presence of salmonellae in the nest box, farm cold room, hatchery truck, or hatchery environment may lead to contaminated eggs. Eggs contaminated in this way can carry salmonellae on the shells or beneath the surface of the shells. The probable mode of natural bacterial contamination of hatching eggs is the cooling of moist, freshly laid eggs from the body temperature of the hen to the air temperature in the presence of contamination on the shell. The hen brings soil and feces into the nest, and these materials have been shown to contain microorganisms, including salmonellae. Eggs laid in wet, dirty nests or on the floor are more likely to be contaminated (Smeltzer et al., 1979). It has been known for over 100 yr that salmonellae can penetrate egg shells, and since that time, numerous studies have demonstrated the penetration and multiplication of salmonellae within the contents of both chicken and turkey hatching eggs. There exists significant variability in salmonellae penetration between eggs Stokes et al., 1956; Humphrey et al., 1989, 1991). Shell quality (Sauter and Petersen, 1974), pH (Sauter et al., 1977), number of pores on an egg shell (Walden et al., 1956), temperature (Graves and Maclaury, 1962), humidity (Gregory, 1948), and vapor pressure (Graves, and Maclaury, 1962) are some of the factors that can have an effect on bacterial penetration of eggs. The outer and inner shell membranes of an egg do offer some protection against bacterial penetration (Baker, 1974), but in spite of this protective effect, several researchers have demonstrated rapid and deep penetration of the egg by various bacteria, including salmonellae. Researchers have demonstrated bacterial penetration in 25 to 60% of inner membranes and in 10 to 15% of albumen in eggs treated on the first day of inoculation (Muira et al., 1964; Humphrey et al., 1989, 1991). Williams et al. (1968) demonstrated that salmonellae penetration of the cuticle and shell occurred almost immediately in some eggs. In fact, in one egg, penetration below both membranes was detected as early as 6 min following shell exposure.

Once bacteria get past the membranes of hatching eggs, there is no way to prevent their further invasion of the egg contents or developing embryo. In addition, the warm temperature of incubation enhances the multiplication of salmonellae. Rizk et al. (1966b) demonstrated that the numbers of salmonellae that have penetrated an egg will greatly increase as the temperature of storage increases, and that the existing conditions during the incubation of a hatching egg promote proliferation of salmonellae.

CONSEQUENCES OF *SALMONELLA* CONTAMINATION OF HATCHING EGGS

Regardless of how an egg becomes contaminated, the resulting possibility for further cross contamination will

be the same when the egg reaches the hatchery (Bailey et al., 1992). Contaminated eggshells have long been thought to lead to the spread of salmonellae in the hatchery. Cox et al. (1990, 1991) found that breeder and broiler hatcheries were highly contaminated with salmonellae. In the broiler hatchery, this contamination was detected on 71% of eggshell fragments, 80% of chick conveyor belt swab samples, and 74% of samples of pads placed under newly hatched chicks to gather feces. Because many eggs coming from a naturally infected flock must be tested to find one that is salmonellae-positive, one must wonder how such an increase in contamination occurs. This question was answered by Cason et al. (1994), who found that eggs inoculated with high numbers of *Salmonella typhimurium* still hatch, because the paratyphoid salmonellae generally do not present a health problem to the chick. Upon hatch, the *Salmonella* is widely disseminated through the hatching cabinet due to fan-forced air. Cason et al. (1994) found that most (>80%) of the chicks hatched in trays both above and below inoculated eggs were positive for an antibiotic-resistant marker strain of *S. typhimurium*. In another study, Cason et al. (1993) showed that eggs carrying salmonellae on the exterior or in shell membranes could lead to contamination of the chick at the pipping of the eggshell. When the embryos were sampled prior to eggshell pipping, no *Salmonella* were detected on body rinses, but after the shell had been breached, 15% of the chickens were externally contaminated and 8% had contaminated yolk sacs.

DEFENSE AGAINST *SALMONELLA* ON HATCHING EGGS

The idea of dipping or washing eggs in disinfectant has been around for a long time (Pritsker, 1941), and a large number of chemicals and protocols of use have been tested in years past. However, the information generated in these earlier studies may be of limited value, because inadequate methods were often used to recover sublethally injured cells of *Salmonella*. Also, when a chemical or chemicals were shown to be effective, too much time had often transpired prior to chemical treatment. For example, 30 min (Bierer et al., 1961; Rizk et al., 1966a), 60 min (Frank and Wright, 1956), 3 h (Gordon et al., 1956), 2 d (Mellor and Banwart, 1965), and 1 to 5 d (Lancaster and Crabb, 1953) were the time intervals between inoculation and application of treatment. We know from the work of Williams and Dillard (1973) that these time intervals are too long to expect the chemical(s) to be effective.

In 1989, the U.S. poultry industry processed 7.5 billion hatching eggs through incubating facilities (Brown, 1989), and that number has now grown to 9 billion. Usually, the invading bacteria do not cause extensive decomposition of the egg, and the chick hatches from the contaminated egg (Maclaury, and Moran, 1959), which results in the establishment of extensive bacterial reservoirs in the commercial hatcheries. Wright et al. (1959) analyzed more than 1,000 samples from 120 commercial hatcheries and found high microbial populations. In recent years, commercial hatcheries have been switching from one chemical or chemical application method to another, believing that the answer lies in either correct choice of chemical or how often or how much to apply. In general, chemicals have had little effect on salmonellae contamination of hatching eggs, because many researchers have demonstrated the presence of salmonellae in commercial hatcheries. Goren et al. (1988) isolated salmonellae from three commercial hatcheries in Europe and demonstrated that the *Salmonella* serotypes originating from the hatchery could subsequently be found on the final product (processed broiler carcass skin). Cox et al. (1990) isolated salmonellae from over 75% of the samples taken in several commercial hatcheries. It has been demonstrated that salmonellae organisms have the ability to persist for long periods of time in commercial hatcheries. Muira et al. (1964) found that over 50% of chick fluff samples were contaminated with salmonellae, and the hatchery fluff kept 4 yr at room temperature still had 1,000 to 1,000,000 viable *Salmonella* cells per gram. That this situation exists even when fumigants and disinfectants recommended for hatchery sanitation are used suggests a need for review of the efficacy of many such recommendations.

Salmonellae-free chickens should be grown and delivered to the processing plant. To accomplish this, salmonellae contamination in fertile hatching eggs and in the hatchery will have to be controlled. To have any chance of controlling this contamination, hatching egg-sanitizing programs will have to be applied at the breeder farm level as soon after the egg is laid as is practically possible.

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